

CLAIMS

WHAT IS CLAIMED IS:

Sub 2

4. The nucleic acid of claim 1, comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:18 or a complementary polynucleotide sequence thereof.

5. The nucleic acid of claim 1, comprising a polynucleotide sequence
5 that has at least about 97% sequence identity to at least one sequence from the group
consisting of SEQ ID NO:1 to SEQ ID NO:18 or a complementary polynucleotide sequence
thereof.

6. The nucleic acid of claim 1, comprising a polynucleotide sequence
that has at least about 98% sequence identity to at least one sequence from the group
10 consisting of SEQ ID NO:1 to SEQ ID NO:18 or a complementary polynucleotide sequence
thereof.

7. The nucleic acid of claim 1, comprising a polynucleotide sequence
that has at least about 99% sequence identity to at least one sequence from the group
15 consisting of SEQ ID NO:1 to SEQ ID NO:18 or a complementary polynucleotide sequence
thereof.

8. The nucleic acid of claim 1, comprising a polynucleotide sequence
that has at least about 80% sequence identity to at least one sequence from the group
consisting of SEQ ID NO:1 to SEQ ID NO:18, or a complementary polynucleotide sequence
thereof, wherein said polynucleotide sequence promotes expression of an operably linked
20 transgene at a level that is greater than the level of expression of the same transgene when
operably linked to a human CMV promoter polynucleotide sequence.

9. The nucleic acid of claim 1, comprising a polynucleotide sequence
comprising a fragment of claim 1 (a), (b), or (c), wherein said fragment promotes expression
of an operably linked transgene at a level that is greater than the level of expression of the
25 same transgene when operably linked to a human CMV promoter polynucleotide sequence.

10. An isolated or recombinant nucleic acid comprising a fragment of one
sequence from the group consisting of SEQ ID NO:1 to SEQ ID NO:18 or a fragment of a

Sub Cwd

complementary polynucleotide sequence thereof, wherein the fragment comprises a unique subsequence.

11. The nucleic acid of claim 10, wherein the fragment promotes the expression of a transgene to which the fragment is operably linked.

5 12. An isolated or recombinant nucleic acid comprising a polynucleotide sequence that hybridizes under highly stringent conditions over substantially the entire length of a polynucleotide sequence of claim 1 (a), (b), (c), or (d).

13. The nucleic acid of claim 12, wherein the highly stringent conditions are selected such that a polynucleotide sequence selected from SEQ ID NO:1 to SEQ ID

10 14. The nucleic acid of claim 12, wherein the highly stringent conditions are selected such that a polynucleotide sequence selected from SEQ ID NO:1 to SEQ ID NO:18 hybridizes to its perfect complement with at least a 5-fold higher signal to noise ratio than for hybridization of the perfect complement to a control nucleic acid comprising a human CMV promoter polynucleotide sequence.

15 14. The nucleic acid of claim 1, comprising a polynucleotide sequence that promotes the expression of an operably linked transgene at a level that differs from the expression level of the same transgene when operably linked to a nucleic acid sequence corresponding to a human CMV promoter polynucleotide sequence.

Sub

15 15. The nucleic acid of claim 14, wherein the transgene is luciferin luciferase, and transgene expression level is determined in an *in vitro* luciferase assay.

20 16. The nucleic acid of claim 14, wherein the transgene is β -galactosidase, the transgene is expressed *in vivo*, and transgene expression level is determined by measuring the serum titer of anti- β -galactosidase antibodies.

17. The nucleic acid of claim 14, wherein the polynucleotide sequence promotes the expression of an operably linked transgene at a level that is higher than the highest expression level of the same transgene when operably linked to a nucleic acid sequence corresponding to a human CMV promoter polynucleotide sequence.

25 18. The nucleic acid of claim 17, wherein polynucleotide sequence promotes the expression of an operably linked transgene at a level that is 2-fold higher than

*Sup
A
Cited by*

the highest expression level of the same transgene when operably linked to a nucleic acid sequence corresponding to a human CMV promoter polynucleotide sequence.

19. The nucleic acid of claim 14, wherein the polynucleotide sequence promotes the expression of an operably linked transgene at a level that is lower than the lowest expression level of the same transgene when operably linked to a nucleic acid sequence corresponding to a human CMV promoter polynucleotide sequence.

20. The nucleic acid of claim 19, wherein polynucleotide sequence promotes the expression of an operably linked transgene at a level that is 2-fold lower than the lowest expression level of the same transgene when operably linked to a nucleic acid sequence corresponding to a human CMV promoter polynucleotide sequence.

21. The nucleic acid of claim 1, wherein the nucleic acid comprises a deletion of one or more nucleotides in a region corresponding to about nucleotides 830-835 or 841-844 of the consensus sequence shown in Figure 8.

22. The nucleic acid of claim 21, wherein the nucleic acid comprises a deletion of nucleotides corresponding to about nucleotides 830-835 or 841-844 of the consensus sequence.

23. The nucleic acid of claim 22, wherein the nucleic acid comprises a deletion of nucleotides corresponding to about nucleotides 830-835 and 841-844 of the consensus sequence.

24. The nucleic acid of claim 1, wherein the nucleic acid comprises a Rhesus monkey CMV promoter polynucleotide sequence at about nucleotide positions 817-863, numbered according to the consensus sequence shown in Figure 8.

25. The nucleic acid of claim 1, wherein the nucleic acid comprises a polynucleotide sequence selected from GACGCCGGAGG and GACGTCGGAG.

26. The nucleic acid of claim 1, wherein the nucleic acid comprises an insertion of a nucleotide, as compared to the human Towne CMV promoter sequence, after nucleotide position 853, numbered according to the consensus sequence shown in Figure 8.

Sub A7

27. The nucleic acid of claim 1, wherein the nucleic acid comprises a deletion of one or more nucleotides in a region corresponding to about nucleotides 684-735 of the consensus sequence shown in Figure 8.

28. The nucleic acid of claim 27, wherein the nucleic acid comprises a 5 deletion of any nucleotides corresponding to about nucleotides 684-735 of the consensus sequence.

29. The nucleic acid of claim 1, wherein the nucleic acid comprises the polynucleotide sequence AAT~~T~~GGCGGGTC. *13*

30. The nucleic acid of claim 1, wherein the nucleic acid does not 10 comprise CMV promoter nucleic acid residues beyond about nucleotide residue 909, numbered according to the consensus sequence shown in Figure 8.

31. The nucleic acid of claim 1, wherein the nucleic acid comprises a polynucleotide sequence comprising nucleic acid residue 1 to about nucleotide residue 930, numbered according to the consensus sequence shown in Figure 8.

32. The nucleic acid of claim 31, wherein the nucleic acid does not 15 comprise CMV promoter nucleic acid residues beyond about nucleotide residue 930, numbered according to the consensus sequence.

33. The nucleic acid of claim 1, wherein the nucleic acid comprises a polynucleotide sequence comprising nucleic acid residue 1 to nucleotide residue 932, 20 numbered according to the consensus sequence shown in Figure 8.

34. The nucleic acid of claim 33, wherein the nucleic acid does not comprise CMV nucleotide residues beyond nucleotide residue 932, numbered according to the consensus sequence shown in Figure 8.

35. The nucleic acid of claim 1, wherein the nucleic acid comprises a 25 deletion of one or more nucleotides in a region corresponding to about nucleotide residues 319-512 of the consensus sequence shown in Figure 8.

SAC
Amend

36. The nucleic acid of claim 35, wherein the nucleic acid comprises a deletion of nucleotides corresponding to about nucleotide residues 319-512 of the consensus sequence.

37. The nucleic acid of claim 1, wherein the polynucleotide sequence 5 comprises SEQ ID NO:21 or a complementary polynucleotide sequence thereof.

38. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:8 (6A8) or a complementary polynucleotide sequence thereof.

39. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:11 (6F6) or a complementary polynucleotide sequence thereof.

10 40. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:6 (3C9) or a complementary polynucleotide sequence thereof.

41. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:9 (6B2) or a complementary polynucleotide sequence thereof.

15 42. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:2 (11E2) or a complementary polynucleotide sequence thereof.

43. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:3 (12C9) or a complementary polynucleotide sequence thereof.

44. The nucleic acid of claims 1, 10 or 12, wherein the polynucleotide sequence is operably linked to a transgene to form an expression cassette.

20 45. The nucleic acid of claim 44, wherein the transgene is a viral gene.

46. The nucleic acid of claim 44, wherein the transgene encodes a polypeptide selected from the group consisting of an immunogen, an immunomodulatory molecule, an antigen, an adjuvant, an allergen, an antibody, a bacterial toxin, a cytokine, a cytokine receptor, and a co-stimulatory molecule.

25 47. The nucleic acid of claim 46, wherein the transgene encodes an antigen selected from the group consisting of a cancer antigen, a hepatitis B surface antigen, a hepatitis A antigen, and a hepatitis C antigen.

48. The nucleic acid of claim 46, wherein the transgene encodes a co-stimulatory molecule comprising a polypeptide that binds to a CD28 or CTLA-4 receptor.

49. A composition produced by the cleaving of one or more nucleic acids of claims 1, 10, or 12, wherein the cleaving comprises mechanical, chemical, or enzymatic cleavage.

50. The composition of claim 49, wherein the cleaving comprises enzymatic cleavage with a restriction endonuclease, an RNase or a DNase.

51. A composition produced by a process comprising incubating one or more nucleic acids of claims 1, 10, or 12 in the presence of deoxyribonucleotide triphosphates and a nucleic acid polymerase.

52. The composition of claim 51, wherein the nucleic acid polymerase is a thermostable polymerase.

53. A method of producing a modified or recombinant nucleic acid comprising mutating or recombining a nucleic acid of claims 1, 10, or 12.

54. The method of claim 53, comprising recursively recombining the nucleic acid with one or more additional nucleic acids.

55. The method of claim 54, wherein the one or more additional nucleic acids promote the expression of an operably linked transgene.

56. The method of claim 54, wherein the recursive recombination is performed *in vitro*.

57. The method of claim 54, wherein the recursive recombination is performed *in vivo*.

58. The method of claim 54, wherein the recursive recombination produces at least one library of recombinant nucleic acids, which library comprises at least one recombinant nucleic acid that promotes the expression of an operably linked transgene.

- Sub 11
Exhibit*
59. The method of claim 53 additionally comprising assaying the modified or recombinant nucleic acid produced by the method for the ability to promote the expression of an operably linked transgene.
60. A nucleic acid library produced by the method of claim 53.
- 5 61. A nucleic acid library comprising two or more nucleic acids of claims 1, 10, or 12.
- 31/2 62. A vector comprising at least one nucleic acid of claims 1, 10, 12 or 44.
63. The vector of claim 62, wherein the vector is an expression vector.
64. The vector of claim 62, wherein the vector is selected from a plasmid, 10 a cosmid, a phage, a virus or fragment thereof, a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC).
- 5 65. A cell comprising the nucleic acid of claims 1, 10, or 12 or the vector of claim 62.
66. The cell of claim 65, wherein the cell comprises a human cell.
- 15 67. A population of cells comprising the library of claims 60 or 61.
68. A composition comprising the nucleic acid of claims 1, 10, or 12 or the vector of claim 62 and a carrier.
69. The composition of claim 68, wherein the excipient is a pharmaceutically acceptable carrier.
- 20 70. The composition of claim 48, wherein the nucleic acid or vector is present in the composition in an amount sufficient to introduce the nucleic acid or vector into cells of a subject, when the composition is administered to the subject.
71. A composition comprising the nucleic acid of claims 1, 10, or 12 or the vector of claim 62 in an amount sufficient to introduce the nucleic acid or vector into 25 cells of a subject, when the composition is administered to the subject.

72. The composition of claims 70 or 71, wherein the amount is sufficient to introduce the nucleic acid or vector into cells of a subject, when the composition is administered to the subject by a route selected from the group consisting of topical administration, injection, implantation, oral administration, buccal, vaginal administration, rectal administration, and inhalation.

73. The composition of claim 75, wherein the composition is administered to the subject by a route selected from the group consisting of intradermal, subdermal, subcutaneous, intramuscular, intravenous, intraperitoneal, and intrathecal.

74. A method of producing a polypeptide, the method comprising:

10 (a) providing a population of cells comprising a nucleic acid of claims 1, 10, or 12 operably linked to a transgene encoding a polypeptide; and

(b) expressing the polypeptide in at least the subset of the population of cells or progeny thereof.

75. The method of claim 74, wherein the population of cells is provided by introducing the nucleic acid operably linked to the transgene into the population of cells.

76. The method of claim 74, further comprising isolating the polypeptide from the cells.

77. The method of claim 74, wherein the cells are in culture.

78. The method of claim 77, comprising expressing the polypeptide by culturing the population or subset of the population of cells or progeny thereof in a nutrient medium under conditions in which the nucleic acid promotes expression of the polypeptide.

79. The method of claim 78, further comprising isolating or recovering the polypeptide from the cells or from the nutrient medium.

80. The method of claim 74, wherein the cells comprise mammalian cells selected from fertilized oocytes, embryonic stem cells, or pluripotent stem cells, the method further comprising generating a transgenic mammal expressing the polypeptide.

81. The method of claim 80, further comprising recovering the polypeptide from the transgenic mammal or a byproduct of the transgenic mammal.
82. The method of claim 74, wherein the cells are *in vivo* in a subject.
83. The method of claim 82, wherein the nucleic acid is introduced into 5 cells in culture, and the cells are subsequently introduced into the subject.
84. The method of claim 82, wherein the nucleic acid is introduced into the cells of the subject by administering the nucleic acid directly to the subject.
85. The method of claim 84, wherein the nucleic acid is administered to the subject by a route selected from the group consisting of topical administration, injection, 10 implantation, oral administration, vaginal administration, rectal administration, and inhalation.
86. The method of claim 85, wherein the nucleic acid is administered to the subject by a route selected from the group consisting of intradermal, subdermal, subcutaneous, intramuscular, ~~intravenous~~, intraperitoneal, and intrathecal.
- 15 87. The method of claim 84, wherein the nucleic acid is administered to the subject by topical administration, injection, or using a gene gun.
88. The method of claim 82, wherein the subject is a human.
89. The method of claim 82, wherein the polypeptide is expressed in an amount sufficient to produce a desired effect in the subject.
- 20 90. The method of claim 89, wherein the desired effect comprises an immunogenic effect, a prophylactic effect, or a therapeutic effect.
91. A nucleic acid of claims 1, 10, or 12 for use in producing an immunogenic effect, a prophylactic effect, or a therapeutic effect in a subject.
92. The nucleic acid of claim 91, wherein the subject is a human.
93. A kit comprising a nucleic acid of claims 1, 10, 12, or 44.

*Sixty
Second*

94. A kit comprising a vector of claims 62 or 63.

95. A database comprising one or more character strings corresponding to a polynucleotide sequence selected from SEQ ID NO:1 to SEQ ID NO:18 or a complementary polynucleotide sequence thereof.

5 96. A database comprising one or more character strings corresponding to a unique subsequence of a polynucleotide sequence selected from SEQ ID NO:1 to SEQ ID NO:18 or a unique subsequence of a complementary polynucleotide sequence thereof.

97. The database of claims 95 or 96, wherein the one or more character strings is recorded in a computer-readable medium.

10 98. A method for manipulating a sequence record in a computer system, the method comprising:

(a) reading a character string corresponding to a polynucleotide sequence selected from SEQ ID NO:1 to SEQ ID NO:18, or a complementary polynucleotide sequence thereof;

15 (b) performing an operation on the character string; and
(c) returning a result of the operation.

99. A method for manipulating a sequence record in a computer system, the method comprising:

(a) reading a character string corresponding to a unique subsequence of a polynucleotide sequence selected from SEQ ID NO:1 to SEQ ID NO:18 or a unique subsequence of a complementary polynucleotide sequence thereof;

(b) performing an operation on the character string; and
(c) returning a result of the operation.

100. The method of claims 98 or 99, wherein the user selects the character string from a database or inputs the character string into the computer system.

25 101. The method of claims 98 or 99, comprising performing one or more operations selected from among: a local sequence comparison, a sequence alignment, a sequence identity or similarity search, a sequence identity or similarity determination, a

SAC

nucleic acid motif determination, a hypothetical translation, a determination of a restriction map, a sequence recombination, or a BLAST determination.

102. The method of claim 101, comprising aligning the selected character string with one or more additional character strings corresponding to a polynucleotide sequence.

103. The method of claim 101, wherein the operation comprises transmitting the character string to a device capable of producing a nucleic acid comprising the polynucleotide sequence corresponding to the character string.